Partial translation of Referenc D

Page 4 (left column, line 16 from the bottom - right column, line 12)

8. Hemokinetics in congestive heart failure model -- effect on urine volume

Eleven adult male or female mongrel dogs (Nakashima Clean Animal Products) weighing from 7 to 14 kg were used. After being introductively anesthetized by thiopental Sodium (25 mg/kg, i.v.), the animals received an intravenous injection of alpha-chloralose (80 mg/kg) and additional infusion (100 mg/body/hr) from the radial vein to maintain anesthetization. A tracheotomy tube was inserted, and artificial respiration (ventilation volume: 20 ml/kg, 20 times/min) was performed by a respirator (SN-480-3, Shinano). Thoracotomy was performed by opening the left 4th intercostal space, the origin of ascending aorta and the left coronary artery descending branch (LAD) were peeled, an inner blood flow probe was provided and the cardiac output was measured by an electromagnetic flowmeter (MF-27, NIHON KOHDEN). A Swan-Ganz catheter (thermo dilution catheter, model TC-504, GOULD) was inserted through the left lateral jugular vein and, right atrial pressure, pulmonary artery pressure and pulmonary artery wedge pressure were measured by a pressure transducer (P 23 IC, GOULD and MPU-0.5A, NIHON KOHDEN). The blood pressure was measured by a pressure transducer (MPU-0.5A, NIHON KOHDEN) connected to the

cannula which was inserted into the right femoral artery. Heart rate was measured by a heart rate meter (AT-600G, NIHON KOHDEN) driven by the output of blood pressure pulse wave. These parameters were continuously recorded on a polygraph (RM-6000, NIHON KOHDEN). Cannulas were inserted into the right and left femoral veins for the administration of test drugs or the drip perfusion of lactated Ringer solution. Urine samples were collected into a graduated cylinder (100 ml) through a silicone catheter (Safeed, Terumo) equipped with a balloon and inserted into the bladder.

Congestive heart failure was induced as follows. Lactated Ringer solution (Otsuka Pharmaceutical Co., Ltd, Lactech ^{R}) was intravenously dripped into the animals, first at the rate of about 20 ml/min over 30 minutes, then the rate was lowered to 10 ml/min and the dripping was continued until the hemodynamics stabilized. 10 minutes after the stabilization, LAD was ligated. Since the hemodynamics would be unstable for about 30 minutes with the risk of arrhythmia being high, carperitide with stepwisely increasing doses (0.1, 0.3, 1 and 3μg/kg/min) was continuously infused for 30 min in each dose. The infusion rate with respect to each dose was 0.03, 0.09, 0.3 and 0.9 ml/min. Control animals received a continuous infusion of the corresponding amount of physiological saline for 30 minutes each. To determine blood carperitide level, 2 ml blood samples were collected through a blood pressure measuring cannula into a 2.5 ml syringe containing 10 mg/ml

EDTA-2Na (Dojindo Laboratories) and 5,000 u/ml aprotinin (trasyrol ^{R} , Bayer) in 0.2 ml distilled water. The samples were centrifuged immediately, and the plasma samples were stored in a refrigerator at -80 ℃. Separately, about 0.5 ml of blood was collected to determine the hematocrit value by a hematocrit tube. The urinary electrolytic concentration was determined as described above under the subtitle "6. Diuretic Action". Carperitide level was determined using a commercial radioimmunoassay kit (human ANP RIA kit, Eiken Chemical, Co., Ltd.).